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14. ABSTRACT

Defined by the presence of malignant cells spreading within intact prostatic ducts and acini, intraductal carcinoma of the prostate (IDC-P) occurs almost exclusively in high Gleason grade and stage tumors and is a consistent independent risk factor for tumor progression and death. Importantly, however, IDC-P is currently systematically under-diagnosed in needle biopsies because it has significant morphologic overlap with another intraepithelial lesion, high grade prostatic intraepithelial neoplasia (HGPIN). We hypothesize that IDC-P represents progression of invasive high-risk prostate carcinoma, and thus is molecularly distinct from HGPIN. Herein, we will conduct an unbiased, three-pronged strategy to definitively identify the molecular signature of IDC-P using a combination of protein, RNA, and DNA-based assays. Then, we will exploit this data to improve pathologic recognition of IDC-P. Here, we report on the use of PTEN/ERG protein as biomarkers of IDC-P in prostate needle biopsy specimens.

15. SUBJECT TERMS
Prostate cancer, intraductal carcinoma, molecular profiling

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1. INTRODUCTION:

Although non-invasive, intraductal carcinoma of the prostate (IDC-P) has long been recognized by pathologists as an extremely high risk feature. Defined by the presence of malignant cells spreading within intact prostatic ducts and acini, IDC-P occurs almost exclusively in high Gleason grade and stage tumors and is a consistent independent risk factor for tumor progression and death in cohorts treated with surgery or radiotherapy. Importantly, however, IDC-P is currently systematically under-diagnosed in needle biopsies because it has significant morphologic overlap with another intraepithelial lesion, high grade prostatic intraepithelial neoplasia (HGPIN). Since HGPIN is a morphologically similar lesion with virtually no prognostic significance, ***we propose that the systematic under-diagnosis of IDC-P in needle biopsies results in the under-recognition of potentially aggressive prostate tumors***. We have found that IDC-P and HGPIN may be readily separable at the *molecular* level, as IDC-P shows an extremely high rate of PTEN loss (84%), a rate even exceeding that seen in invasive high Gleason grade tumors. In contrast, HGPIN never shows loss of this tumor suppressor. Although our preliminary candidate gene data is compelling, *the current challenge is to systematically elucidate the molecular profile of IDC-P, a study which will not only yield additional clinically useful markers of this specific lesion but also elucidate the molecular features of an extremely high risk subset of prostate tumors*. The aims of the current study are to: 1) *Validate* PTEN and ERG as specific, clinically applicable markers of IDC-P, using a combination of immunohistochemistry and fluorescence *in situ* hybridization (FISH); 2) *Profile* the gene expression signature of IDC-P and systematically compare it to HGPIN, identifying additional candidate markers for distinguishing the two lesions; and 3) *Integrate* IDC-P into the molecular landscape invasive carcinoma, both at the gene expression and genomic levels, using a combination of bioinformatics, targeted next generation sequencing and copy number variation analysis.

2. KEYWORDS:

Prostatic carcinoma, intraductal carcinoma, high grade prostatic intraepithelial neoplasia, molecular profiling

3. OVERALL PROJECT SUMMARY:

Task 1: *Validate* PTEN and ERG as specific molecular markers of IDC-P (months 4-24, allowing for 3 month regulatory review of IRB protocols)

1a. Assess PTEN/ERG protein status via immunohistochemistry (IHC) in 40 biopsies each of: isolated IDC-P meeting current morphologic criteria, IDC-P with concurrent invasive carcinoma, and age-matched cases of isolated HGPIN (months 4-10)

1b. Assess whether PTEN protein loss via IHC predicts for subsequent cancer diagnosis and/or adverse pathologic outcomes in 40 cases of isolated intraductal lesions that did not meet current morphologic criteria for IDC-P (months 4-24).

1c. Validate PTEN IHC assays by correlating with *PTEN* fluorescence *in situ* hybridization (FISH) in 45 IDC-P lesions on tissue microarray (months 6-14).

Progress on Task 1: Expedited IRB approval for use of tissue specimens was received (11/15/13, "Development and Validation of Prognostic and Predictive Biomarkers in Human Prostate Tumor Specimens" JHU IRB # NA_00091198) and tasks 1a and 1b have been completed. Separate or combined immunostains for PTEN and ERG were applied to de-identified prostate biopsies containing morphologically-identified intraductal carcinoma, PIN or borderline intraductal proliferations more concerning than PIN, but falling short of morphologic criteria for intraductal carcinoma. Intraductal carcinoma occurring with concurrent invasive tumor showed the highest rate of PTEN loss, with 76% (38/50) lacking PTEN and 58% (29/50) expressing ERG. Of biopsies containing isolated intraductal carcinoma, 61% (20/33) showed PTEN loss and 30% (10/33) expressed ERG (**Table 1**). Of the borderline intraductal proliferations, 52% (11/21) showed PTEN loss and 27% (4/15) expressed ERG (**Figure 1**). Of the borderline cases with PTEN loss, 64% (7/11) had carcinoma in a subsequent needle biopsy specimen, compared to 50% (5/10) of PTEN-intact cases. In contrast, none of the PIN cases showed PTEN loss or ERG expression (0/19). Taken together, on needle biopsy, PTEN loss is common in morphologically identified intraductal carcinoma yet is very rare in high grade PIN. Borderline intraductal proliferations, especially those with PTEN loss, have a high rate of carcinoma on resampling. If confirmed in larger prospective studies, these results suggest that PTEN and ERG immunostaining may provide a useful ancillary assay to distinguish intraductal carcinoma from high grade PIN in this setting. In terms of task 1c, we are currently optimizing the PTEN FISH assay for performance on tissue microarrays (TMAs) and will hybridize the IDC-P TMA in the near future.

Task 2: *Profile* the gene expression signature of IDC-P and compare it to that of HGPIN (months 8-36).

2a. Use laser capture microdissection (LCM) to obtain epithelial cells from morphologically-identified IDC-P and PIN occurring with concurrent Gleason 8 tumors and perform DASL and subsequent differential gene expression analysis to establish respective molecular signatures (months 12-36)

2b. Validate the top 3 promising candidate markers for distinguishing IDC-P from HGPIN at the RNA and protein levels using immunohistochemistry (IHC) and RNA *in situ* hybridization (ISH) on specimens collected in Task 1 (months 24-36).

Progress on Task 2: We have identified over 50 cases of isolated PIN and IDC-P, each and are currently selecting cases for sectioning in preparation for LCM. This work is ongoing and LCM is expected to begin in the next 4-6 months. Task 2b has not yet begun.

Task 3: *Integrate* IDC-P into the molecular landscape of invasive carcinoma (months 18-36).

3a. integrate the expression data for HGPIN and IDC-P into pre-existing, identically-collected datasets for high and low grade invasive tumors using Correspondence at the Top (CAT) plot analysis—supervised by Dr. Luigi Marchionni (months 18-28).

3b. Use the Ampliseq Comprehensive Cancer Panel to compare exomic sequences of 409 oncogenes/tumor suppressor genes in IDC-P with the sequences from the concurrent invasive cancer within each case (n=20 samples total) and confirm a subset of detected mutations using Taqman mutation detection assays (months 18-36).

3c. Use the Nanostring nCounter Cancer Copy Number Assay to compare copy number profile across 86 genes in IDC-P with those in concurrent invasive tumors (n=20 samples total) (months 18-36).

Progress on Task 3: This task has not yet begun

4. KEY RESEARCH ACCOMPLISHMENTS:

- IRB approval at JHU for this human tissue study has successfully be obtained.
- PTEN and ERG have been studied by immunohistochemistry as biomarkers of IDC-P, and appear to be useful to distinguish this lesion from PIN on prostate biopsies.
- PTEN loss predicts for worse outcomes in borderline intraepithelial lesions where the differentiation diagnosis is HGPIN vs IDC-P.

5. CONCLUSION:

Our most recent results suggest that PTEN and ERG can serve as useful immunohistochemical biomarkers of IDC-P and help to distinguish this aggressive lesion from indolent HGPIN in prostate biopsy specimens. We are preparing to do additional expression analyses in order to select additional biomarkers and to do genomic analyses to integrate IDC-P into the molecular landscape invasive carcinoma.

6. PUBLICATIONS, ABSTRACTS AND PRESENTATIONS:

A manuscript describing Tasks 1a and 1b has been submitted for publication.

7. INVENTIONS, PATENTS AND LICENSES: None

8. REPORTABLE OUTCOMES: None

9. OTHER ACHIEVEMENTS:

10. REFERENCES:

None.

11. APPENDICES:

None.

FIGURES AND TABLES:

Table 1: Rate of PTEN loss and ERG expression in spectrum of intraepithelial prostate proliferations

Intraepithelial lesion	PTEN loss	ERG expression
Intraductal carcinoma with concurrently sampled invasive carcinoma	**76%** (38/50)	**58%** (29/50)
Isolated intraductal carcinoma	**61%** (20/33)	**30%** (10/33)
Borderline intraductal proliferations	**52%** (11/21)	**27%** (4/15)
PIN with concurrently sampled invasive carcinoma	**0%** (0/7)	**0%** (0/7)
Isolated PIN	**0%** (0/12)	**0%** (0/12)

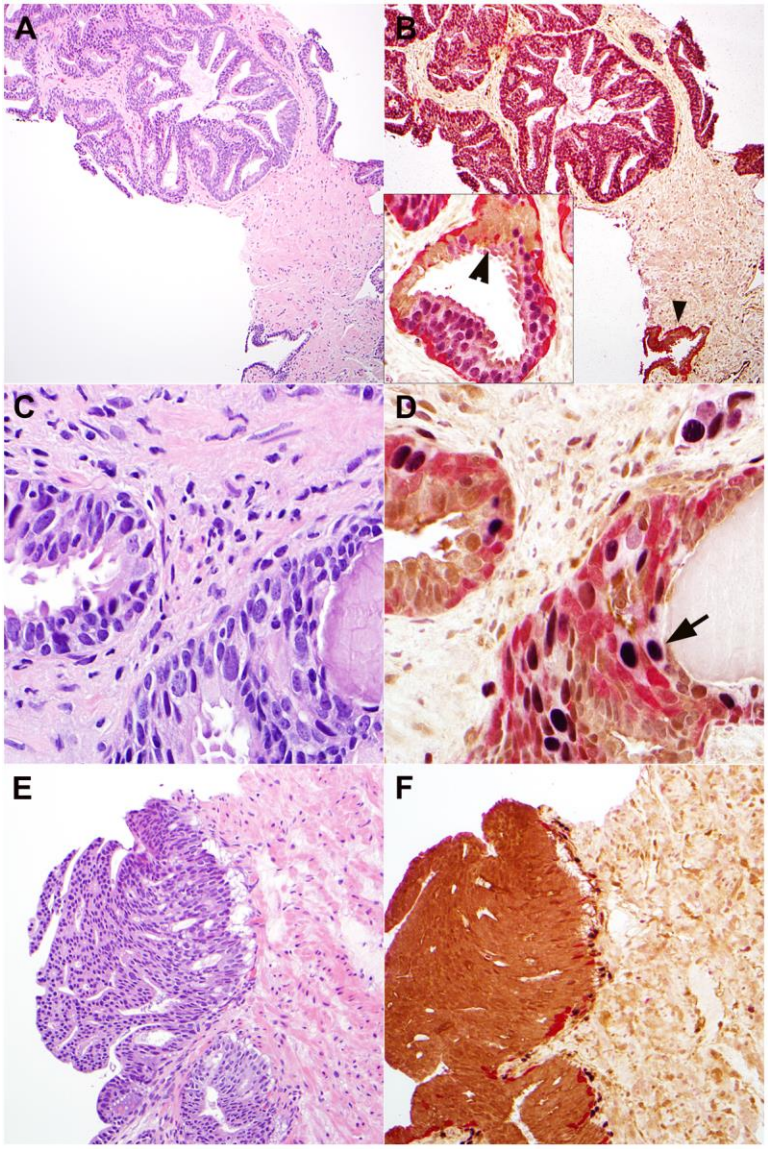

Figure 1: PTEN and ERG expression in borderline intraepithelial proliferations more concerning the PIN, but insufficient for a diagnosis of intraductal carcinoma using current morphologic criteria (A) Borderline proliferation with loose cribriform architecture, unusual for PIN, but insufficient for diagnosis of intraductal carcinoma (100x magnification). (B) Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (A) demonstrates PTEN loss relative to adjacent benign cells (inset shows involved gland from different area of core; arrowhead demonstrates nearby benign gland) and diffuse expression of ERG. (C) Borderline proliferation with substantial cytologic atypia (arrow) but lacking sufficient atypia to qualify as intraductal carcinoma (630x magnification). (D) Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (C) demonstrates pagetoid spread of PTEN-negative, ERG-positive cells (arrow). (E) Borderline proliferation (200x magnification) with dense cribriform architecture which is highly suspicious for intraductal carcinoma but insufficiently represented at the edge of the needle core. (F) Quadruple immunostain for PTEN

(brown), ERG (purple) and basal cells (red) on case in (E) demonstrates retention of PTEN and lack of ERG expression in the proliferation.